REMARKS

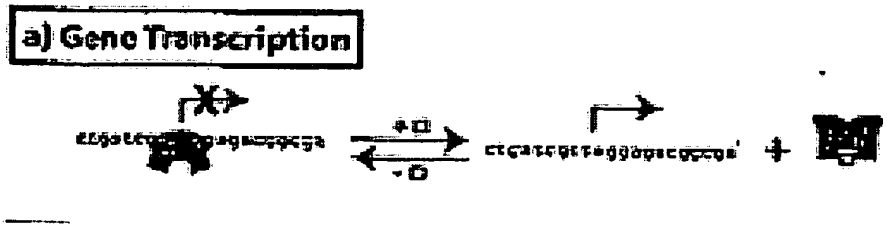
Claims 1 – 8, 14, 26, 30, 32, 38, 39, 41, and 45 – 47 are pending in the application. Claims 1 – 8, 14 and 45 - 47 are under examination. Claims 1 and 7 have been amended. Claims 26, 30, 32, 38, 39, and 41 have been canceled as being directed to non-elected inventions. No new claims have been added. No new matter has been added by virtue of the amendments, support being found in the specification and in the claims as originally filed.

Objections

The Examiner has maintained his objection to the disclosure. The Examiner alleges that "there are only 2 nucleotide sequences in Figure 3A rather than 3 sequences of SEQ ID Nos. 39 – 41." (Office Action, p.2). Applicants disagree.

Applicants refer the Examiner to the prior response dated March 29, 2007. Applicants pointed out to the Examiner the Amendment submitted on 3/24/2005 in response to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide and/or Amino Acid Sequence Disclosures mailed by the Office on January 24, 2005. In the Amendment dated 3/24/2005, Applicants amended the specification to recite SEQ ID Nos. 39 – 41 in Figure 3A. Accordingly, Applicants direct the Examiner to Figure 3A, below, which "shows regulation of gene transcription using a fusion molecule according to one aspect of the invention (SEQ ID NOs 39 – 41 respectively in order of appearance)." (Amendment of 3/24/2005, page 9). Applicants point out that there are in fact **three nucleotide sequences in Figure 3A,** as shown in the Figure below.

Figure 3A



SEQ ID NOs 39 – 41 are listed in the specification as follows, and clearly correspond to the sequences listed in Figure 3A:

39 ctgatcc

40 gagacggcga

41 ctgatcgcta ggagacggtg c

Accordingly, Applicants respectfully request withdrawal of the objection.

Claim Rejections- 35 U.S.C. § 102 (b)

Claims 1 – 8 and 14 were rejected under 35 U.S.C. § 102 (b) as being anticipated by Siegel et al., (Methods in Enzymology. 2000. Vol. 327, p.249 – 259). The Examiner argues the Siegel reference teaches that "the polymerase chain reaction (PCR) works well for inserting GFP into signal transduction proteins and that channel proteins, in particular, are surprisingly tolerant to GFP insertions." (Office Action, p.3). Applicants respectfully traverse the rejection.

The instant claims recite a method for assembling a modulatable fusion molecule. The method as instantly claimed comprises inserting randomly an insertion sequence into an acceptor sequence, wherein the insertion sequence and the acceptor sequence each comprise a state, and thereby generating a fusion molecule, wherein the fusion molecule comprises a new state.

In order to anticipate a claim, each and every element of the claim must be found in a single reference. This is discussed in the Manual of Patent Examining Procedure § 2131:

> "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). "The identical invention must be shown in as complete detail as is contained in the . . . claim." Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989). The elements must be arranged as required by the claim, but this is not an ipsissimis verbis test, i.e., identity of terminology is not required. In re Bond, 910 F.2d 831, 15 USPQ2d 1566 (Fed. Cir. 1990).

The teachings of the Siegel reference do not anticipate the claimed invention. Specifically, the Siegel reference does not teach or suggest a method for assembling a modulatable fusion molecule that comprises **inserting randomly** an insertion sequence into an acceptor sequence, and thereby generating a fusion molecule.

As pointed out by the Examiner, the Siegel reference teaches that "in the case of Shaker...GFP can be inserted at the N terminal, at the C terminal, and also at a variety of internal sites." (Office Action, p.3). The Examiner argues that "it appears that Siegel does suggest insert(ing) GFP into signal transduction protein and channel protein randomly at various different insertion sites and found that they are surprisingly tolerant to GFP insertions." (Office Action, p.3). Applicants disagree.

The Seigel reference "describe(s) a modified green fluorescent protein (GFP) fused to a voltage sensitive K+ channel." (p.249). The Siegel reference does not teach insertion of GFP into signal transduction or channel proteins at **random** sites, but rather teaches insertion of GFP at sites based on structural and functional information. Applicants direct the Examiners attention, for

example, to Figure 1, which shows the "GFP based sensors of cell signaling." (p.251). In Figure 1 (A)

> GFP is fused in frame into the middle of a signal transduction protein (detector) so that conformational rearrangements in the detector perturb the fluorescence of GFP. The GFP has been sensitized to the rearrangements of the detector protein by deletion of the last eight residues in the C terminal, which are disordered in the crystal structure. (p.251)

From the description in Figure 1A, it is clear that the GFP is fused in the signal transduction protein in a manner such that voltage dependent rearrangements in the channel are detected by the fluorescent protein. The fusion is based on a functional requirement. Accordingly, **this is not a random insertion**.

In Figure 1B, Siegel describe "a chimeric protein that is a modified GFP fused in frame at a site just after the sixth transmembrane segment (Fig. 1B) of the voltage-activated Shaker channel." (p.252). Siegel clearly point to a preferred site of fusion and, further indicate that "the detailed description and characterization of this sensor protein have been described previously" (p.252) in their own work, which describes "the fusion of a modified green fluorescent protein (GFP) into a voltage-sensitive K+ channel so that voltage-dependent rearrangements in the K+ channel would induce changes in the fluorescence of GFP." (Siegel and Isacoff. Neuron. 1997 Oct;19(4):735-41. Abstract). Again, Applicants point out that the fusion or insertion of GFP is based on a functional requirement, and as such is not random.

Siegel et al. teaches insertion of GFP based on the structural information. For example, at page 256, Siegel teach

> (t)he tolerance of many proteins to GFP insertion is probably due to the structure of GFP in which the N and C termini emerge in close proximity to one another on the same side of the barrel. (p.256)

Thus, insertion of GFP is based on structural knowledge of the protein. Accordingly, **this is not a random insertion**.

Applicants respectfully request withdrawal of the rejection and allowance of the claims.

Claims 1 – 8 and 14 were rejected under 35 U.S.C. § 102 (b) as being anticipated by Lacatena et al (PNAS. 1994. Vol. 91, pp.10521 – 10525). The Examiner argues that Lacatena "teaches using TnphoA, a transposon probe for protein export signals, to generate hubeta2AR-phoA fusion protein in vivo by transposition of TnphoA into the hubeta2AR gene in PUC18." (Office Action, p.3). Applicants respectfully traverse the rejection.

The instant claims recite a method for assembling a modulatable fusion molecule. The method as instantly claimed comprises inserting randomly an insertion sequence into an acceptor sequence, wherein the insertion sequence and the acceptor sequence each comprise a state, and thereby generating a fusion molecule, wherein the fusion molecule comprises a new state.

In order to anticipate a claim, each and every element of the claim must be found in a single reference. The teachings of the Lacatena reference do not anticipate the claimed invention. Specifically, the Lacatena reference does not teach or suggest a method for assembling a modulatable fusion molecule that comprises inserting randomly an insertion sequence into an acceptor sequence, and thereby generating a fusion molecule, wherein the fusion molecule comprises a new state. The Lacatena reference does not teach random insertion.

The Lacatena reference is a topological analysis of the human beta2 adrenergic receptor in E.coli using hubeta2AR-PhoA fusions to determine the proteins' topology in the bacterial membrane and the requirements for correct insertion into the membrane. (p.10521). The Lacatena reference teaches that "a combination of in vivo and in vitro techniques were used to isolate gene fusions encoding proteins with various lengths of hubetaAR." (p.10522). As shown in Figure 2, Lacatena describes "gene fusions encoding proteins with various lengths of huBetaAR from the N terminus joined to PhoA at the C terminus." (p.10522). Lacatena do not teach or suggest a method for assembling a modulatable fusion molecule that comprises inserting randomly an insertion sequence into an acceptor sequence, but rather use a PhoA fusion approach

to isolate gene fusions encoding different lengths of human betaAR joined to PhoA. The fusions described by Lacatena are **only** between the N-terminal region of human BetaAR and C-terminal PhoA, and thus do not involve random insertion as taught in the instant claims.

Applicants respectfully request withdrawal of the rejection and allowance of the claims.

Claims 1 – 8 and 14 were rejected under 35 U.S.C. § 102 (b) as being anticipated by Anderson et al (USPN 6,596,485 B2; the '485 reference herein). The Examiner argues that "Anderson teaches 'in a preferred embodiment the peptide library is fully randomized with no sequence or constants at any position.'" (Office Action, p.4). Applicants respectfully traverse the rejection.

Instant claim 1 is as described above.

The '485 reference "provides fusion proteins comprising a random peptide fused to green fluorescent protein (GFP)." (see, e.g. col.1, line 10, or col. 2, line 60) As pointed out by the Examiner, the '485 reference merely teaches "inserting a peptide into GFP at numerous different positions." (Office Action, p.5). **The insertion of the peptide taught by the '485 reference is not random**. In fact, the '485 reference clearly teaches that various factors were used to select sites for insertion. For example, the '485 reference teaches that temperature was used to choose loops for peptide insertion:

> Figure 1 depicts the crystal structure of GFP showing the temperature factors used to pick some of the loops for internal insertion of random peptides. (col. 2, lines 34 – 36).

Nowhere does the '485 reference teach or suggest a method for assembling a modulatable fusion molecule that comprises **inserting randomly** an insertion sequence into an acceptor sequence, and thereby generating a fusion molecule**, wherein the fusion molecule comprises a new state**.

Accordingly, Applicants respectfully request withdrawal of the rejection and allowance of the claims.

Claims 1 – 8 and 14 were rejected under 35 U.S.C. § 102 (b) as being anticipated by Doi et al (FEBS Letters, Vol.453, p.305 – 307). The Examiner alleges that "random mutagenesis of the fusion GFP:Bla gene using error-prone PCR to obtain a plasmid library encompasses random insertion of an insertion sequence into an acceptor sequence. Applicants respectfully disagree.

Instant claim 1 is as described above.

The Doi reference is directed to the construction of "generic GFP based sensors (using) a screening method combined with insertional gene fusion technique." (p.305). The Doi reference describes a fusion protein in which the BLIP gene is inserted in a **specific position** in the surface loop of the GFP protein. As described on page 305:

> the BLIP gene minus the signal sequence (codons 1 – 36) (is) inserted between the PBAD promoter from *Escherichia coli* JM109 and the *rrnB* T1T2 terminator from pEOR.

The Examiner argues that "random mutagenesis of the fusion GFP:Bla gene using error prone PCR to obtain a plasmid library encompasses random insertion of an insertion sequence into an acceptor sequence." (Office Action, p.5). It seems that the Examiners argument is misplaced, as the methodology taught by the Doi reference and described above clearly does not involve any random insertion. Applicants respectfully request that the Examiner clarify this point if the rejection is maintained.

Accordingly, Applicants respectfully request withdrawal of the rejection and allowance of the claims.

Claim Rejections- 35 U.S.C. § 112, second paragraph

Claims 45 – 47 were rejected under 35 U.S.C. § 112, second paragraph for alleged indefiniteness. The Examiner argues "the phrase 'one or more method selected from nuclease treatment...chemical treatment or radiation treatment' in claim 45 is vague and renders the claim indefinite." (Office Action, p.6). Applicants respectfully traverse the rejection.

Applicants have amended the claim to recite the language suggested by the Examiner.

Accordingly, Applicants respectfully request withdrawal of the rejection and allowance of the claims.

Claim Rejections- 35 U.S.C. § 112, first paragraph

Claims 45 – 47 were rejected under 35 U.S.C. § 112, first paragraph for allegedly failing to comply with the written description requirement. The Examiner argues that "the phrase 'one or more of a method selected from nuclease treatment, mechanical shearing, chemical treatment or radiation treatment' is considered new subject matter." Applicants respectfully traverse the rejection.

The Examiner is allegedly "unable to find the support for the phrase set forth above in the specification or claims." (Office Action, p.7).

Applicants point the Examiner first to paragraph [0021] of the published Application which reads:

> The method may further comprise the step of digesting the second nucleic acid with a nuclease such as DNase I, S1 nuclease, mung bean nuclease, a restriction endonuclease, or a combination thereof, shearing the second nucleic acid (e.g., mechanically), or otherwise treating the second nucleic acid to introduce breaks (e.g., exposing the nucleic acid to chemical agents and/or radiation).

Applicants next point the Examiner to paragraph [0130] of the published Application which reads:

> In addition to digestion by nucleases (e.g., DNAse, S1, exonucleases, restriction endonucleases and the like), other methods for introducing breaks in sequences can be used. For example, mechanical shearing, chemical treatment, and/or radiation can be used. Generally, the method for introducing breaks is not intended to be limiting.

Similar support can be found in paragraph [0140].

Applicants point the Examiner to paragraph [0131] of the published Application which reads:

> As shown in FIG. 2C, a key step in the creation of these libraries is the digestion of the gene fragments with a **3' to 5' exonuclease** such as Exonuclease III (Exo III) under conditions (e.g., low temperature or in the presence of NaCl) such that the digestion rate is controlled to .about.10 bases/minute or less.

Applicants next point the Examiner to paragraph [0177] of the published Application which reads:

> In one aspect, incremental truncation is used to engineer a conditional heterodimer. In the example for implementing this approach, shown in FIGS. 6A-B, two overlapping fragments of a gene encoding a polypeptide whose state is to be switched are cloned into vectors. Incremental truncation libraries **from the 3' end of the 5' fragment and the 5' end of the 3' fragment** are prepared using time-dependent exonuclease digestion

The specification as filed contains clear support related the subject matter of claims 45 – 47.

Applicants respectfully request withdrawal of the rejection and allowance of the claims.

CONCLUSIONS

For the reasons provided, Applicant submits that all claims are allowable as written and respectfully requests early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record.

A three month extension of time for response is requested.

The Director is hereby authorized to charge any credits or deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to Deposit Account No. 04-1105.

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Respectfully submitted,

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